CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
ACETAMIPRID

Chemical Code # 5762, Tolerance # 52854
SB 950 # N/A
10/1100

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, no adverse effects
Chronic toxicity, dog:	No data gap, no adverse effects
Oncogenicity, rat:	No data gap, no adverse effects
Oncogenicity, mouse:	No data gap, no adverse effects
Reproduction, rat:	No data gap, no adverse effects
Teratology, rat:	No data gap, no adverse effects
Teratology, rabbit:	No data gap, no adverse effects
Gene mutation:	No data gap, no adverse effects
Chromosome effects:	No data gap, possible adverse effect
DNA damage:	No data gap, no adverse effects
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.

NOTE: Some studies list the a.i. as "NI-25". This is noted in the year 2000 Farm Chemicals Handbook as a former Nippon Soda code number for Acetamiprid.

All record numbers through 177488 (Document No. 52854-114) were examined. This includes all records indexed by DPR as of 10/1100.

In the one-liners below:
** indicates an acceptable study.
Bold face indicates a possible adverse effect.

File name: T183632
Revised by Aldous on 10/11/00.

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

52854-044 175547 Hatch, R. C., "Two year dietary toxicity and oncogenicity study in rats", MPI Research, Inc., 9/28/99 (second revised final report). Study # 449-015. Fifty Crl:CD®BR rats/sex/group were dosed in diet with 0, 160, 400, or 1000 ppm acetamiprid (purity 99.7%) for 2 years in a guideline combined study. An additional 10/sex/group were sacrificed at 1 year. Estimated mean compound intakes over the full study were 7.1, 17.5, and 46.4 mg/kg/day for males, and 8.8, 22.6, and 60.0 mg/kg/day for females. Chronic NOEL = 160 ppm (7.1 and 8.8 mg/kg/day for males and females, respectively). Dose-related findings at 400 ppm were significantly reduced body weight (females), and hepatocellular hypertrophy and vacuolation (highly statistically significant in males only). Additional 1000 ppm effects included significant body weight decrements in males, general increases in water consumption in both sexes, decreased triglyceride levels in high dose females, decreased thymic weight in females (without associated histopathology), slight hepatocellular hypertrophy in females, and increased incidence of papillary microconcretions in kidneys of females. The latter was slightly elevated in degree at 400 ppm also, representing a very weak response compared to liver effects cited as bases of the NOEL. Study is **acceptable, with **no adverse effects**. Aldous, 7/18/00.

CHRONIC TOXICITY, RAT

(See Combined, Rat: above)

CHRONIC TOXICITY, DOG

**52854-039 175541 Auletta, C. S., "A chronic (12-month) oral toxicity study of NI-25 in the dog via dietary administration, Pharmaco LSR, Inc., 4/29/98. Study No. 92-3117. Beagle dogs, 4/sex/group, were dosed with 0, 240, 600, or 1200 ppm NI-25 (Acetamiprid), 99.57% purity, in diets for 1 year in a guideline chronic study. Estimated mean achieved dose levels were 9, 20, and 55 mg/kg/day in increasing dose groups of males, and 9, 21, and 61 mg/kg/day in females. NOEL = 600 ppm (20 mg/kg/day), based on relatively persistent body weight decrements in both sexes. Food consumption was also substantially reduced in high dose dogs, particularly during the early weeks of the study. One finding, thymic interstitial hemorrhage, was observed in 3 high dose females, but not in any other dogs of either sex. Extent of lesions in two of these females were of "marked" degree: both of these dogs also displayed grossly red discoloration of mediastinal tissue, corresponding to hemorrhage at that location. There were no correlated findings within this study, nor did the long-term rat and mouse studies (Record Nos. 175547 and 175542) indicate any analogous findings. Similarly, the subchronic dog study (Record No. 175534) found no treatment effects on this lesion up to 2000 ppm in diet. DPR review thus determined that the thymic findings in the chronic study were likely to be incidental, despite the unusual incidence distribution of this thymic lesion. Report is acceptable, with no adverse effects. Aldous, 7/13/00.

ONCOGENICITY, RAT

(See Combined, Rat: above)

ONCOGENICITY, MOUSE

**52854-040 175542 Goldenthal, E. I., "18-month dietary oncogenicity study in mice", MPI Research, Inc., 9/17/99 (2nd revised report). Laboratory Project ID 449-016. Crl:CD-1®(ICR)BR mice, 50/sex/group, received Acetamiprid, purity 99.38%, in diet at 0, 130, 400, or 1200 ppm for 1.5 years in a guideline oncogenicity study. An additional 10/sex/group received these doses prior to sacrifice at 12 months. Estimated average doses were 20, 66, and 186 mg/kg/day in males, and 25, 76, and 215 mg/kg/day in females. NOEL = 130 ppm (20 and 25 mg/kg/day in males and females, respectively).

Findings at 400 ppm included a very low incidence of liver centrilobular hypertrophy (1/sex affected), modest but statistically significant body weight decrements in males, and small but statistically significantly increased relative liver weights in females. Especially the body weight and liver hypertrophy findings were more pronounced at 1200 ppm. Other findings at 1200 ppm included decreased food consumption with associated clinical signs of "decreased defecation", slightly reduced prostate weights, reduced adrenal weights in 400 and 1200 ppm (terminal sacrifice females only), and increased incidence of chronic progressive nephropathy (terminal sacrifice females only, only "trace" degree of change, equivocal as a treatment effect). Study is acceptable, with no adverse effects. Aldous, 7/21/00.

REPRODUCTION, RAT

**52854-043 175546 Trutter, J. A., "Two-generation reproduction study with NI-25 in rats (Reproduction and fertility effects)", Covance Laboratories Inc., 11/13/99 (amended report date). Study ID: Covance 6840-108. Acetamiprid (99.9% purity) was administered in diet at 0, 100, 280, and 800 ppm to 26/sex/group in a standard reproduction study (1 litter period/generation), with the addition of estrous cycle determinations (vaginal smear analyses); sperm count, morphology, and mobility determinations; developmental landmark determinations (pinna unfolding, upper incisor eruption, and eye opening in all pups, and vaginal opening or preputial separation in prospective parental rats during the pre-mating phase); plus Day 0 anogenital measurements of F2 pups. Parental toxicity NOEL = 280 ppm (18 and 21 mg/kg/day in M and F, respectively), based on reduced food consumption and reduced body weights in both sexes of both generations. Modest but statistically significant decrements in absolute brain weights in F1 adult males and females were also noted at 800 ppm. Reproductive NOEL = 280 ppm, based on substantial decrements in pup weights (both generations) and also on reduced pup survival throughout the lactation period in F2 pups. The study is acceptable, with no adverse effects. Aldous, 8/4/00.

TERATOLOGY, RAT

** 52854-041 175544 Nukui, T. and Y. Fujii "Acetamiprid – Teratogenicity study in rats", Odawara Research Center, Nippon Soda Co., Kanagawa, 9/29/97 (amended version). Lab Project ID No. G-0829. Twenty-four Crj: CD ™ (SD) dams/group were dosed on days 6-15 by gavage with 0, 5, 16, or 50 mg/kg/day acetamiprid [99.46%, in aqueous suspension (5% gum arabic, 0.01% Tween 80)] using a standard teratology study design. Maternal NOEL = 16 mg/kg/day, based on decreased food consumption, decreased body weight gain, and slightly elevated liver weights. Developmental NOEL = 16 mg/kg/day, based on the skeletal variation: shortening of the 13th rib (litter incidences of 1, 3, 1, and 8 in controls through high dose groups, respectively). Study is acceptable, with no adverse effects. Aldous, 8/10/00.

TERATOLOGY, RABBIT

** 52854-042 175545 Nukui, T. and Y. Fujii, "Acetamiprid – Teratogenicity study in rabbits," Odawara Research Center, Nippon Soda Co., Kanagawa, 9/29/97 (amended version). Lab Project ID No. G-0830. Seventeen mated NZW rabbits/group were dosed on days 6-18 by gavage with 0, 7.5, 15, or 30 mg/kg/day acetamiprid [99.46%, in aqueous suspension (5% gum arabic, 0.01% Tween 80)] using a standard teratogenicity study design. High dose does consumed significantly less diet than any other groups during the first two days of dosing. By day 4 of treatment, there was no difference in food intake. Body weight gains of high dose females were slightly (but not significantly) smaller than those of other groups over the course of treatment. There were no other indications of maternal toxicity. Developmental NOEL = 15 mg/kg/day, based on fused thoracic vertebral arches and fused ribs in 2 fetuses (2 litters), vs. no such findings in other groups. The latter was not considered to be a basis for a NOEL by investigators, who noted that this observation was not statistically significant. This DPR review recommends that the equivocal developmental finding should be considered as a possible treatment effect. Considering the marginal extent of the changes at a maternally toxic dose level, and noting also presence of a conservative NOEL at 50% of this dose level, the data do not warrant designating a "possible

adverse effect." Acceptable. C. Aldous, 8/14/00.

GENE MUTATION

**52854-045 175549 Kanaguchi, Y., "Acetamiprid – reverse mutation study on bacteria," Odawara Research Center, Nippon Soda Co., Kanagawa, Dec. 1, 1997. Laboratory Project ID: G-0831. *Salmonella typhimurium* strains TA100, TA1535, TA98, and TA1537, and *E. coli* strain WP2 uvrA were used in reverse mutation tests with and without S9. There were 3 plates/trial, and 2 trials with S9 and 2 trials without S9 at each concentration of acetamiprid (99.2% purity) in 2x steps from 313 µg/plate to 5000 µg/plate, using 20-minute pre-incubation followed by plating. There was no increase in reversion frequencies at any concentration. Positive controls were functional. Acceptable, with no adverse effects. Aldous, 9/21/00.

**52854-046 175558 Adams, K., "Acetamiprid: mammalian cell mutation assay," Huntingdon Life Sciences, Ltd., Huntingdon, England, 2/24/98. Laboratory Project ID: NOD 006. CHO-K1-BH_4 cells were tested for forward mutation at functionally hemizygous HPRT locus following incubation for 4 hr in the presence and absence of S9. Aliquots of 10^6 viable cells were incubated over a 7-day expression time prior to trypsinization and seeding portions into three 60 mm plates (200 cells/plate, non-selective medium) and into five 100 mm plates (2×10^5 cells/plate, selective medium). Acetamiprid, purity 99.9%, was evaluated at 4 levels in each test (2 independent tests with and without S9). Highest concentrations used in a given test were based on associated cytotoxicity assays. For tests without S9, high concentrations were 3500 and 4000 µg/ml. For tests with S9, high concentrations were 2000 and 2750 µg/ml. There were no increases in mutant frequencies in acetamiprid groups. Positive controls were functional. Acceptable, with no adverse effects. Aldous, 9/8/00.

CHROMOSOME EFFECTS

NOTE: One *in vitro* study below was positive at high concentrations. Two *in vivo* studies were negative. At present, there is considered to be a possible adverse effect based on the *in vitro* study.

**52854-045 175552 Murli, H., "Mutagenicity test on NI-25 in an *in vivo* mouse micronucleus assay," Hazleton Washington, Inc. 8/26/98. HWA Study No. 15901-0-455. CD-1 mice, 5/sex/group/interval, were dosed with 0, 20, 40, or 80 mg/kg acetamiprid (99.57% purity) by gavage in 0.5% CMC at 24, 48, or 72 hr before sacrifice. An additional 5/sex were dosed at 80 mg/kg as available replacement animals. Positive controls, cyclophosphamide at 80 mg/kg, were treated (5/sex) at 24 hr pre-sacrifice only. PCE's from femur bone marrow were evaluated for micronuclei. Two males at 80 mg/kg were found dead immediately after dosing, and all other 80 mg/kg mice experienced tremors. A total of five males and 4 females from the 80 mg/kg acetamiprid group died within 5 hr of treatment. There was no treatment-related increase in micronuclei at any dose or interval tested. Cyclophosphamide controls were functional. Acceptable. No adverse effect. Aldous, 5/21/00.

**52854-045 175555 Durward, R., "NI-25: Metaphase analysis in the rat bone marrow *in vivo*," Safepharm Laboratories Limited, Derby, UK, 2/19/98. SPL Project No. 235/017R2. CD rats, 5/sex/dose/interval, were dosed by gavage with 250 mg/kg acetamiprid, purity 99.46% (Arachis oil BP vehicle) at pre-sacrifice intervals of 6, 24, or 48 hr. There were corresponding vehicle controls, plus cyclophosphamide (50 mg/kg) as a positive control (only the 24 hr interval for the latter). Rats were treated with colchicine 2 hr before sacrifice. Marrow cells from femurs were isolated, then fixed in MeOH/acetic acid. Portions of each suspension were dried and stained with Giemsa. Fifty spreads per rat were examined for chromosomal aberration effects. There was no increase in chromosomal aberrations. Acceptable with some deficiencies noted in worksheet. Aldous, 9/13/00.

****52854-046 175556** Kanaguchi, Y., "Acetamiprid – chromosomal aberration study in Chinese Hamster Ovary (CHO) cells," Odawara Research Center, Nippon Soda Co., Kanagawa, Dec. 1, 1997. Laboratory Project ID G-0800. A fibroblast cell line isolated from Chinese hamster ovary was used to evaluate chromosomal aberration potential. Acetamiprid levels were 175, 350, 700 µg/ml without S9 and 337.5, 675, 1350 µg/ml with S9. Concentrations used were based on suppression of mitotic indices in

range-finding tests. Incubation times were selected to accommodate cell cycle delays at higher dose levels. Acetamiprid levels of 175, and 700 µg/ml without S9 were associated with modest but statistically significant ($p < 0.05$) increases in chromosomal aberrations. The latter were of equivocal toxicological importance. Acetamiprid at 675 and 1350 µg/ml in the presence of S9 clearly caused increases in chromosomal aberrations (significant, $p < 0.01$ and $p < 0.001$, respectively). The relevance of chromosomal aberrations at these high concentrations is unclear, since neither osmolality nor possible pH effects were evaluated in this study. Acceptable, with a possible adverse effect. Aldous, 9/21/00.

52854-047 175562 Curry, P. T., "Mutagenicity test on IM-1-4 in the *in vivo* mouse micronucleus assay," Covance (Vienna, VA), 6/29/98. Covance Project ID No. 18981-0-455OECD. CD-1 mice, 6/sex/group/interval, were dosed with 0, 175, 350, or 700 mg/kg IM-1-4 (minor metabolite of acetamiprid, 99.6% purity) by gavage (deionized water vehicle) at 24, 48, or 72 hr before sacrifice. Positive controls, cyclophosphamide at 80 mg/kg, were treated (6/sex) at 24 hr pre-sacrifice only. PCE's from bone marrow (femur or tibia) were evaluated for 5 mice per sex/dose/time point for micronuclei. Treatment did not increase micronuclei formation. Cyclophosphamide controls were functional. Valid supplemental study. No adverse effect. Aldous, 9/21/00.

DNA DAMAGE

52854-045 175554 San, R. H. C., and J. E. Sly, "Unscheduled DNA Synthesis (UDS) test with mammalian liver cells *in vivo*," Microbiological Associates, Inc., 10/3/97. Report No. G97AG26.381. Male Sprague-Dawley rats were dosed by gavage with 0, 75, 150, or 300 mg/kg acetamiprid, purity 99.9%, either 2-4 hours before sacrifice, or 12-16 hours before sacrifice. Positive controls (dimethylnitrosamine) were treated in parallel. Three rats/treatment/interval were evaluated for UDS. The high dose caused no deaths, but elicited lethargy and tremors in one rat and remarkably darkened livers in all rats. Although it appeared that a somewhat higher dose could have been survivable, marked clinical signs of toxicity were observed at 400 mg/kg, whereas all rats died at 1250 mg/kg in a range-finding test. Based on range-finding study survival and toxicity data, the high dose selected for the UDS study is acceptable. There was no indication of treatment effects at dose levels tested. Unacceptable, with no adverse effect. Upgradeable with isolated hepatocyte viability data. Aldous, 9/21/00.

**52854-046 175557 Ham, A. L., "Genotoxicity test on NI-25 in the assay for unscheduled DNA Synthesis in rat liver primary cell cultures with a confirmatory assay," Hazleton Washington, Inc., 8/26/98. HWA Study No. 15901-0-447R. Hepatocytes from F344 males were used (one rat donor per trial) in two trials. Although cultures were prepared over a wide dosage range, both trials used 6 concentrations of approximately 10, 25, 50, 100, 250, and 500 µg/ml for UDS assays. Higher concentration of 1000 µg/ml led to survivals of 15% and 32%, which were below the 50% minimal survival standard for a viable treatment level, according to protocol. Positive controls, 2-AAF, were functional. Both trials were negative for UDS with acetamiprid using autoradiography. Acceptable, with no adverse effects. Aldous, 9/21/00.

NEUROTOXICITY STUDIES

031; 175533; “Acetamiprid: Neurotoxicity to Rats by Acute Oral Administration” (Hughes, E.W., Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, Project Identity RNP/509, 11/3/97). 818. Acetamiprid (Batch No.:NFG-02, purity=99.9%), suspended in 0.5% sodium carboxymethylcellulose, was administered by gavage in a single dose to 10 Crl:CD BR rats per sex per dose at dose levels of 0 (vehicle only), 10, 30 and 100 mg/kg. No mortalities occurred. A treatment-related increase in the frequency and severity of body tremors during FOB arena observations were observed in males at 30 and 100 mg/kg and in females at 100 mg/kg on Day 0 (six hours post-dose). A treatment-related decrease in mean locomotor activity was observed in males at 30 and 100 mg/kg and in females at 100 mg/kg on Day 0. Also, gait abnormalities, a decrease in body temperature, and dilated pupils were observed in both sexes at 100 mg/kg on Day 0. No treatment-related effects were observed during FOB assessments conducted on days 7 and 14. Microscopic examination revealed no treatment-

related abnormalities. **Possible adverse effect**: treatment-related tremors. NOEL (M)=10 mg/kg (based on increased frequency and severity of body tremors and decreased locomotor activity); NOEL (F)=30 mg/kg (based on increased frequency and severity of body tremors, dilated pupils, decreased body temperature, and decreased locomotor activity). **Acceptable**. (Corlett and Leung, 8/25/00)

031; 175532; "Acetamiprid: Dose Range Finding Neurotoxicity to Rats by Acute Oral Administration (including determination of time to peak effect)" (Hughes, E.W., Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, Identification No. RNP 510/970145, 10/28/97). Acetamiprid (Batch No.:NFG-02, purity=99.9%), suspended in 0.5% sodium carboxymethylcellulose, was administered by gavage in a single dose to 3 Crl:CD BR rats per sex per dose at dose levels of 10, 50 and 100 mg/kg. Tremors in 2 males and 2 females and dilated pupils in 2 females were observed at 100 mg/kg. An increase in body tremors and a decrease in arousal (arena observations) during FOB assessments were observed in both sexes at a greater frequency 5 hours post-dose than at 1/2 hour or 2 hours post-dose. Greater decreases in both body weight and body temperature during FOB assessments were observed in both sexes 5 hours post-dose than at 1/2 hour or 2 hours post-dose. Necropsy revealed no treatment-related abnormalities. NOEL cannot be assigned (no negative control group used in the study). **Supplemental** (only 3 animals per sex per dose level were used and no negative control group was included in the study). (Corlett, 8/22/00)

038; 175540; "Acetamiprid: Neurotoxicity to Rats by Dietary Administration for 13 Weeks" (Hughes, E.W., Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England, Project Identity RNP/511, 11/3/96). 827. Acetamiprid (Batch No.:NFG-02, purity=99.9%) was admixed to the diet at dose levels of 0 (untreated diet), 100, 200, 800, or 1600 ppm (for males, 0, 7.4, 14.8, 59.7, and 118 mg/kg/day, respectively, and for females, 0, 8.5, 16.3, 67.6, and 134 mg/kg/day, respectively) and fed to 10 Crl: CD BR rats per sex per dose for 13 weeks. No mortalities occurred. Cageside observations revealed no clinical signs. Treated-related decreases in mean body weight, mean body weight gain, and mean cumulative food consumption were observed in both sexes at 800 and 1600 ppm. During Week 4 FOB observations, treatment-related brown nasal staining was observed in both sexes at 1600 ppm. Motor activity assessments revealed no treatment-related effects. Microscopic examination of the nervous system revealed no treatment-related abnormalities. **No adverse effects**. NOEL (M)=14.8 mg/kg/day (200 ppm) and (F)=16.3 mg/kg/day (200 ppm) (based on decreases in mean body weight, mean body weight gain, and mean cumulative food consumption). **Acceptable**. (Corlett and Leung, 8/30/00)

SUBCHRONIC STUDIES

(Oral)

033; 175535; "Acetamiprid: Thirteen-Week Dietary Subchronic Toxicity Study in Mice" (Nukui, T. and Ikeyama, S., Toxicology Laboratory, Odawara Research Center, Nippon Soda Co., Ltd., Kanagawa, Japan, Project No. G-0769, 9/29/97). 821. Acetamiprid (31-1359 (NI-25)) (Lot No.591001-7 (Tox-470), purity=99.2%) was admixed to the diet at dose levels of 0 (untreated diet), 400, 800, 1600, or 3200 ppm (for males, 0, 53.2, 106.1, 211.1, and 430.4 mg/kg/day, respectively, and for females, 0, 64.6, 129.4, 249.1, and 466.3 mg/kg/day, respectively) and fed to 10 Crj: CD-1^{TM} (ICR) mice per sex per dose for a period of 13 weeks. 2 males and 2 females at 3200 ppm died during the study. Tremor was observed in 5 females at 3200 ppm. Treated-related decreases in mean body weight at 1600 and 3200 ppm and mean food consumption at 3200 ppm were observed in both sexes. A treated-related increase in mean relative liver weight was observed in both sexes at 800, 1600, and 3200 ppm. Microscopic examination revealed centrilobular hepatocellular hypertrophy and depletion of fat from the adrenal cortex in both sexes at 3200 ppm. **Possible adverse effect**: tremor in high dose females. NOEL (M)= 53.2 mg/kg/day (400 ppm) and (F)= 64.6 mg/kg/day (400 ppm) (based on an increase in mean relative liver weight). **Acceptable**. (Corlett, 9/15/00)

034; 175536; "Acetamiprid: Thirteen-Week Toxicity Study in Rats" (Nukui, T. and Ikeyama, S., Toxicology Laboratory, Odawara Research Center, Nippon Soda Co., Ltd., Kanagawa, Japan, Project No. G-0768, 9/29/97). 821. Acetamiprid (31-1359 (NI-25)) (Lot No.31-0223-HY (Tox-447), purity>99%) was admixed to the diet at dose levels of 0 (untreated diet), 50, 100, 200, 800, or 1600 ppm (for males, 0, 3.1, 6.0, 12.4, 50.8, and 99.9 mg/kg/day, respectively, and for females, 0, 3.7, 7.2,

14.6, 56.0, and 117.1 mg/kg/day, respectively) and fed to 10 Crj: CD^{TM} (SD) rats per sex per dose for a period of 13 weeks. No mortalities occurred. No treatment-related clinical signs were observed. A treated-related decrease in mean body weight was observed in both sexes at 800 and 1600 ppm. A treated-related increase in mean relative liver weight was observed in both sexes at 800 and 1600 ppm. Microscopic examination revealed centrilobular hepatocellular hypertrophy in both sexes at 800 and 1600 ppm. **No adverse effects**. NOEL (M)= 12.4 mg/kg/day (200 ppm) and (F)= 14.6 mg/kg/day (200 ppm) (based on a decrease in mean body weight, an increase in mean relative liver weight, and centrilobular hepatocellular hypertrophy). **Acceptable**. (Corlett, 9/11/00)

032; 175534; "A Subchronic (3-Month) Oral Toxicity Study of NI-25 in the Dog Via Dietary Administration" (Auletta, C.S., Bio/Dynamics, Inc., East Millstone, NJ, Study No. 91-3727, 30/98). 821. NI-25 (Lot No.NNI-02, purity=99.46%) was admixed to the diet at dose levels of 0 (untreated diet), 320, 800, or 2000 ppm (for males, 0, 13, 32, and 58 mg/kg/day, respectively, and for females, 0, 14, 32, and 64 mg/kg/day, respectively) and fed to 4 beagle dogs per sex per dose for a period of 3 months. No mortalities occurred. No treatment-related clinical signs were observed. A treated-related decrease in mean body weight was observed in both sexes at 2000 ppm. Macroscopic and microscopic examinations revealed no toxicologically significant effects. **No adverse effects**. NOEL (M/F)= 32 mg/kg/day (800 ppm) (based on a decrease in mean body weight). **Acceptable**. (Corlett, 10/2/00)

(Dermal)

037; 175539; "21-Day Dermal Toxicity Study in Rabbits with Acetamiprid" (Trutter, J.A., Covance Laboratories Inc., Vienna, VA, Laboratory Study Identification: Covance 6224-236, 10/30/97). 822. Acetamiprid (NI-25) (Lot No. NFG-02, purity=99.9%), moistened with deionized water, was applied to the clipped skin of 5 New Zealand White rabbits per sex per dose at dose levels of 0 (deionized water only), 100, 500, or 1000 mg/kg/day for 6 to 6.5 hours per day, 5 days per week for at least 3 weeks. No mortalities occurred. No treatment-related clinical signs or dermal irritation were observed. No treatment-related body weight, hematological, or serum chemistry effects were observed. Macroscopic and microscopic examinations revealed no treatment-related abnormalities. **No adverse effects**. NOEL (M/F, systemic and dermal)=1000 mg/kg/day based on no effects at HDT. **Acceptable**. (Corlett, 10/4/00)

METABOLISM

**52854-048 175566 Tanoue, T. and H. Mori, "^{14}C-NI-25 - Metabolism study in rats (A summary report)", (a summary of several metabolism studies, comprising 52854-048 to 52854-050), 09/25/97. This summary appropriately concluded that studies indicate that acetamiprid is efficiently absorbed and rapidly excreted, primarily in urine. Fecal elimination was primarily via the bile. Although a small percentage of administered dose was excreted as acetamiprid, most compound was N-demethylated, and much of the material lost the side chain, yielding chain residues and 6-chloronicotinic acid, some of latter becoming conjugated with glycine. By day 4, tissue residue levels were uniformly low. Whereas some differences were noted due to route, dose level, or duration of exposure, there are no apparent reasons for concern about disposition of acetamiprid. Collective metabolism studies are acceptable. Aldous, 8/31/00.

52854-050 175486 Premkumar, N. D., C. Y. Guo, and S. S. Vengurlekar, "Absorption, distribution, metabolism, elimination, and pharmacokinetics after chronic dosing of [^{14}C]-NI-25 in rat," ABC Laboratories, Inc., Columbia, MO, 3/24/95. Study No. 42207. SD rats were dosed by gavage with non-labeled acetamiprid (>99.9% purity) or with labeled acetamiprid (radiopurity of 97.2%, label on the pyridine ring) to evaluate urinary and fecal excretion patterns and tissue residues over time. All acetamiprid dose levels were 1 mg/kg/day. Three groups (Groups I-III) containing 3/sex/interval were all dosed daily for 15 days with labeled acetamiprid, then sacrificed at 1 hr, 10 hr, or 96 hr after the final dose, respectively. These rats were examined for tissue levels of ^{14}C at termination, and also blood samples were taken from the 96-hour group on days 1, 3, 7, and 15 at one hour after respective dosings. Two groups containing 5/sex/interval (Groups IV and V) were dosed for 14 days with unlabeled acetamiprid, followed by labeled acetamiprid on day 15. These were used to obtain tissue levels at 96 hr after the last dosing (Group IV) or to follow blood levels during serial samplings over 48 hours after last

dosing (Group V). Maximal blood levels were obtained at 2.8 hr after dosing (M or F), and estimated elimination half-life was 4.42 hr (M) or 5.56 hr (F). In general, this rapid elimination was almost twice as much in urine as in feces. Male elimination in feces was consistently a few percent higher than for corresponding females. Tissue levels were initially highest in GI tract, liver, and kidneys, with 96 hr levels several-fold lower than peak levels for all tissues. Rats dosed with labeled acetamiprid daily had generally higher tissue levels 96 hr after the last treatment compared to rats who received labeled acetamiprid only on day 15, suggesting that a small but measurable accumulation of some comparatively stable residues had occurred. This study is valid for its purpose. Aldous, 8/15/00.

52854-050 175487 Premkumar, N. D. and C. Y. Guo, "[^{14}C]-NI-25 – Biliary excretion in rat," ABC Laboratories, Inc., Columbia, MO, 3/17/95. Study No. 42206. Sprague-Dawley rats were prepared with cannulae for gastric intubation, for bile collection from the common bile duct, and for biliary fluid replacement into the duodenum. Three males and four females with patent cannulae were administered 1.02 to 1.07 mg/kg of acetamiprid, each in a single gavage dose. Non-labeled acetamiprid was >99.9% purity, to which was added labeled acetamiprid containing ^{14}C in positions 2 and 6 of the pyridine ring, of radiopurity of 97.2%. Primary observed parameters included bile collection for periods ending at 3 hr, 6 hr, 12 hr, 24 hr, and 48 hr; urinary and fecal excretion at intervals ending at 24 hr and 48 hr; and residual radiolabel in liver, GI tract, and residual carcass at 48-hr termination. Collected bile accounted for 18.1% to 20.8% of administered dose: in most cases the bulk of radiolabel was collected between 3 hr and 12 hr. Feces accounted for an average of 6.7% (M) and 5.8% (F) of administered dose. This indicated efficient absorption. Since Record No. 175486 had found that about 30% of administered dose was excreted in feces, it is apparent that biliary elimination is a significant route for the rat. About 60-64% of administered dose was found in urine and cage washings (presumed to be primarily urine), suggesting that urinary excretion exclusive of re-circulated biliary products was the main mode of excretion. For both urinary and fecal excretion, residues were about 2 to 3-fold higher in the first 24-hr collection than in the second 24-hr period. This study is valid for its purpose. Aldous, 8/15/00.

52854-049 175570 Tanoue, T. and H. Mori, "^{14}C-NI-25 - Metabolism study in rats," Nisso Chemical Analysis Service Co., Ltd., Odawara Laboratory, Kanagawa, Japan, 3/31/97. Study No. NCAS 2-94. Crj:CD rats, 5 to 9/sex/group, were dosed by gavage with either 1 or 50 mg/kg of ring-labeled acetamiprid for analysis of blood levels, tissue distribution, excretion rates, and quantitative analyses of metabolites. Similar groups were dosed with *cyano* carbon-labeled acetamiprid for analysis of blood levels, excretion rates, and quantitative analyses of metabolites. Limited studies (blood levels and excretion rates) followed iv administration of ring-labeled acetamiprid. Oral dosing of 1 mg/kg led to maximal blood levels within 2 hr of treatment in either sex. Under these conditions, elimination half-life estimates were 6 to 11 hr. Oral dosing of 50 mg/kg was associated with a minor delay in maximal blood levels (3-7 hr), and with longer elimination half-lives (8 and 15 hr in M and F, respectively). In all cases, elimination was predominantly urinary: generally over 70% of label was found in urine within 24 hr of dosing. Day 1 elimination in feces did not exceed 12% of administered dose. Tissue concentrations tended to be highest in kidneys, liver, thyroids, and adrenals. Levels decreased sharply in these organs as well as in all other tissues over 4 days, regardless of dose level, position of the label, or route of administration. Acetamiprid was present in urine at about 3-5% in most cases (6-7% in high dose rats). The N-dealkylated metabolite (IM-2-1) was more abundant (urinary output containing13-24% of administered label). For ring-labeled acetamiprid, the most abundant metabolite found in urine was 6-chloronicotinic acid (IC-0, accounting for 24-28% of administered radioactivity). These same chemicals were the most common identifiable fecal metabolites, most of which did not account for over 1% of administered dose. Study is valid for its portion of metabolism data requirements. Aldous, 8/28/00.

52854-048 175568 Tanoue, T. and H. Mori, "^{14}C-NI-25 - Metabolism study in rats" (Qualitative and quantitative analysis of metabolites in Group C), Nisso Chemical Analysis Service Co., Ltd., Odawara Laboratory, Kanagawa, Japan, 3/27/97. [In-life phase of study was at Analytical Bio Chemistry Laboratories, Inc. (Columbia, MO): urinary and fecal samples were then sent to Odawara Laboratory for analyses]. Study No. NCAS 95-108. The in-life portion of the study was reviewed separately [52854-050 175486 Premkumar, N. D., C. Y. Guo, and S. S. Vengurlekar, "Absorption, distribution, metabolism, elimination, and pharmacokinetics after chronic dosing of [^{14}C]-NI-25 in rat," ABC Laboratories, Inc., Columbia, MO, 3/24/95. ABC Laboratories Study No. 42207.] The present record reports metabolite determinations in urinary and fecal samples, and allows comparison of this multiple-dose study with single-dose studies reported in 52854-049 175570. Exposure conditions for the present

study were detailed in Record No. 175486 (see that 1-liner for descriptions of the 6 treatment groups). The essential and unique element to the present report is the identification of metabolites in urine and feces. There were no apparent sex differences in metabolite patterns after 14 days of dosing with unlabeled acetamiprid, followed by a single treatment with ring-labeled acetamiprid on day 15. Under these conditions, Day 1 urinary percentages of total administered doses were: unchanged acetamiprid (3.4% and 3.1%, M & F), the N-dealkylated metabolite (IM-2-1) (10.8% and 9.9%, M & F), 6-chloronicotinic acid (IC-0) (8.0% and 3.3% in M and F), and the IC-0-glycine conjugate (9.3% and 6.8% in M and F). Consistent with single-dose studies, this study did not find other major metabolites. This multiple-dose study found appreciably more IC-0-glycine conjugate than did the several single-dose 1 mg/kg studies (where total urinary IC-0-glycine yields never exceeded 4.0% and 1.4% in M & F, respectively: see Record No. 175570). Non-conjugated IC-0 and IM-2-1 comprised somewhat lesser portions of administered dose than in the single-dose studies. Otherwise there were no remarkable differences between urinary metabolite patterns following multiple exposures compared to single dose studies. Major urinary metabolite levels decreased at least 3-fold in males from day 1 to day 2, but clearance ran somewhat more slowly in females over days 2-4. Fecal excretion, measured only for day 1, found the same metabolites to predominate as for urinary excretion (except IC-0-glycine, which was not abundant enough to quantify). Fecal residues always represented smaller percentages of administered dose than corresponding urinary residues, although the percentages of administered dose excreted in feces in this study on day 1(26.2% and 21.7% in M and F) were at least twice the percentages found in the single-dose studies. This supplemental study is a valid component of the series of metabolism studies. Aldous, 8/30/00.

SPECIAL (NON-FIFRA, SUPPLEMENTAL) STUDIES

52854-025 175498 Mochizuki, N., and Y. Fujii, "Acetamiprid: pharmacological studies in experimental animals," Nippon Soda Co., Ltd., Odawara Research Center, Kanagawa, Japan. Dec. 11, 1997. Project No. G-0832. Acetamiprid, 99.46%, in 20%/80% DMSO/saline for i.p. and i.v. injections, in 20%/80% DMSO/distilled water for gavage, or in DMSO vehicle for *in vitro* studies. Primary or characteristic significant findings were:
Crj:ICR mice: General activity/behavior: NOEL = 5 mg/kg i.p. (vocalizations at 10 mg/kg, decreased alertness, weakness, abnormal posture, tremor, decreased spontaneous activity, and staggering gait at 20 mg/kg, 1/3 deaths at 30 mg/kg). Prolonged pentobarbital sleeping time (NOEL = 10 mg/kg, LOEL = 20 mg/kg). Decreased time to pass charcoal in g.i. tract (NOEL/LOEL = 20 mg/kg and 40 mg/kg).
Crj:CD(SD) rats: Reduced urinary volume (NOEL/LOEL = 10 mg/kg and 20 mg/kg i.p.). No change in AChE in plasma up to MTD of 20 mg/kg.
Std:Hartley guinea pigs (for isolated ileum preparation): transient contraction/relaxation effect (NOEL/LOEL = 10^{-5} g/ml and 10^{-4} g/ml).
Kbs:NZW rabbits: General activity/behavior: NOEL = 10 mg/kg i.v. (decreased spontaneous activity, muscle tone, alertness; incoordination, convulsions, increased respiratory rate at 30 mg/kg, deaths at 60 mg/kg). Decreased blood pressure (NOEL/LOEL = 1 mg/kg and 3 mg/kg).
These are supplemental studies not required under FIFRA. Aldous, 8/31/00.

Gene Mutation Studies on Metabolites

52854-045 175550 Mochizuki, N. and Y. Kanaguchi, "IM-1-2 – reverse mutation study on bacteria," Odawara Research Center, Nippon Soda Co., Kanagawa, 9/30/97. Laboratory Project ID: G-964. *Salmonella typhimurium* strains TA100, TA1535, TA98, and TA1537, and *E. coli* strain WP2 uvrA were used in reverse mutation tests with and without S9. There were 3 plates/trial, and 2 trials with S9 and 2 trials without S9 at each concentration of IM-1-2 (a potential acetamiprid metabolite, >99.9% purity) in 2x steps from 313 µg/plate to 5000 µg/plate, using 20-minute pre-incubation followed by plating. There was no increase in reversion frequencies at any concentration. Positive controls were functional. Valid supplemental study, with no adverse effects. Aldous, 9/21/00.

52854-045 175551 Mochizuki, N. and Y. Kanaguchi, "IM-1-4 – reverse mutation study on bacteria," Odawara Research Center, Nippon Soda Co., Kanagawa, 9/30/97. Laboratory Project ID G-940. *Salmonella typhimurium* strains TA100, TA1535, TA98, and TA1537, and *E. coli* strain WP2 uvrA

were used in reverse mutation tests with and without S9. There were 3 plates/trial, and 2 trials with S9 and 2 trials without S9 at each concentration of IM-1-4 (a minor acetamiprid metabolite, 99% purity) in 2x steps from 313 µg/plate to 5000 µg/plate, using 20-minute pre-incubation followed by plating. The highest concentration generally caused growth inhibition, but there was no such effect at lower levels. There were no treatment-related changes in reversion frequencies. Positive controls were functional. Valid supplemental study, with no adverse effects. Aldous, 9/21/00.

52854-047 175561 Mochizuki, N. and Y. Kanaguchi, "IM-2-1 – reverse mutation study on bacteria," Odawara Research Center, Nippon Soda Co., Kanagawa, 9/30/97. Laboratory Project ID G-932. *Salmonella typhimurium* strains TA100, TA1535, TA98, and TA1537, and *E. coli* strain WP2 uvrA were used in reverse mutation tests with and without S9. There were 3 plates/trial, and 2 trials with S9 and 2 trials without S9 at each concentration of IM-2-1 (a major acetamiprid metabolite, >99.9% purity) in 2x steps from 313 µg/plate to 5000 µg/plate, with 20 minutes pre-incubation before plating. There was no growth inhibition at these levels. There were no treatment-related changes in reversion frequencies. Positive controls were functional. Valid supplemental study, with no adverse effects. Aldous, 9/21/00.

52854-047 175563 Mochizuki, N. and Y. Kanaguchi, "IC-0 – reverse mutation study on bacteria," Odawara Research Center, Nippon Soda Co., Kanagawa, 9/30/97. Laboratory Project ID G-942. *Salmonella typhimurium* strains TA100, TA1535, TA98, and TA1537, and *E. coli* strain WP2 uvrA were used in reverse mutation tests with and without S9. There were 3 plates/trial, and 2 trials with S9 and 2 trials without S9 at each concentration of IC-0 (a major acetamiprid metabolite, 99.4% purity) in 2x steps from 313 µg/plate to 5000 µg/plate, with 20 minutes pre-incubation before plating. There was no growth inhibition or precipitation except at 5000 µg/plate. Growth inhibition was evident in TA98 plates with and without S9 at 5000 µg/plate. Precipitation was seen in all strains at 5000 µg/plate with S9, and usually growth inhibition was also seen at this level with S9. There were no treatment-related changes in reversion frequencies. Positive controls were functional. Valid supplemental study, with no adverse effects. Aldous, 9/21/00.

52854-047 175559 Cifone, M. A., "Mutagenicity test on IM-1-4 in the CHO/HGPRT forward mutation assay," Covance Laboratories, Inc., 6/29/98. Covance Study No. 6840-106. IM-1-4, a very minor metabolite of acetamiprid in the rat, 99.7% purity, was tested in CHO-K1-BH_4 cells in presence and absence of S9. Cultures containing about 4×10^6 cells were exposed for about 4 hr to test article, followed by 7 days incubation to allow expression of mutations (1.5×10^6 cells/dish: 2 dishes per treatment), with periodic subculturing to promote logarithmic growth. At the end of this expression period, each culture was distributed into 12 dishes, each containing about 2×10^5 cells. These were incubated 7 days in selective medium (containing 24 µM thioguanine), then fixed with methanol and stained with Giemsa. High dose levels were limited by cytotoxicity at about 3000 µg/ml, and tests evaluated six concentrations up to this level with and without S9. The test with S9 was clearly negative, however the test without S9 presented statistically significant increases at the lowest and highest concentrations tested (250 and 3000 µg/ml), but not at intermediate levels. The investigator considered this result to be negative, since a criterion for a "positive" response was that the mutant frequency achieve at least 15×10^{-6} in order to compensate for inherent variability for this assay. The high dose in this case was very cytotoxic (survival and cell population growth were <10% of control), and should be considered of questionable value in this circumstance. Also, the finding at the lowest concentration appears to be spurious, since the next four higher concentrations were not notably cytotoxic, yet showed no sign of mutagenicity. Valid supplemental study. No adverse effects. Aldous, 9/21/00.

52854-047 175560 Mochizuki, N. and Y. Kanaguchi, "IM-0 – reverse mutation study on bacteria," Odawara Research Center, Nippon Soda Co., Kanagawa, 9/30/97. Laboratory Project ID G-949. *Salmonella typhimurium* strains TA100, TA1535, TA98, and TA1537, and *E. coli* strain WP2 uvrA were used in reverse mutation tests with and without S9. There were 3 plates/trial, and 2 trials with S9 and 2 trials without S9 at each concentration of IM-0 (a minor acetamiprid metabolite, 99.14% purity) in 2x steps from 313 µg/plate to 5000 µg/plate, with 20 minutes pre-incubation before plating. There was no growth inhibition at these levels. There were no treatment-related changes in reversion frequencies. Positive controls were functional. Valid supplemental study, with no adverse effects. Aldous, 9/21/00.

SUBCHRONIC STUDIES ON METABOLITES

036; 175538; "IM-0- Thirteen-Week Dietary Subchronic Toxicity Study in Rats" (Nukui, T. and Ikeyama, S., Toxicology Laboratory, Odawara Research Center, Nippon Soda Co., Ltd., Kanagawa, Japan, Project No. G-0889, 11/28/97). IM-0 (CPA) (Lot No.NK-3266 (Tox-563), purity=98.94%) was admixed to the diet at dose levels of 0 (untreated diet), 160, 800, 4000, or 20000 ppm (for males, 0, 9.9, 48.9, 250.1, and 1246.6 mg/kg/day, respectively, and for females, 0, 11.1, 55.9, 275.9, and 1173.7 mg/kg/day, respectively) and fed to 10 Crj: CD™ (SD) rats per sex per dose for a period of 13 weeks. No mortalities occurred. No treatment-related clinical signs were observed. Treated-related decreases in mean body weight and mean food consumption at 20000 ppm were observed in both sexes. A treated-related increase in mean relative kidney weight was observed in both sexes at 20000 ppm. Microscopic examination revealed kidneys with treatment-related esinophilic intranuclear inclusions in the proximal tubular epithelium in males at 4000 and 20000 ppm and in females at 20000 ppm. **No adverse effects**. NOEL (M)= 48.9 mg/kg/day (800 ppm) and (F)= 275.9 mg/kg/day (4000 ppm) (based on treatment-related microscopic findings in the kidney). **Acceptable**. (Corlett, 9/20/00)

114; 177488; "13-Week Dietary Subchronic Toxicity Study with IM-1-4 in Rats" (Ivett, J.L., Covance Laboratories Inc., Vienna, VA, Study No. 6840-102, 2/1/99). IM-1-4 (Lot No.NK-97127, purity=99.6%) was admixed to the diet at dose levels of 0 (untreated diet), 200, 600, 1800, or 5400 ppm (for males, 0, 12.8, 36.5, 112.2, and 319.3 mg/kg/day, respectively, and for females, 0, 15.6, 44.6, 135.6, and not determined (due to loss of data) mg/kg/day, respectively) and fed to 10 Crl: CD®BR rats per sex per dose for a period of 13 weeks. No mortalities occurred. No treatment-related clinical signs were observed. A treated-related decrease in mean body weight at 5400 ppm was observed in both sexes. Treated-related increases in mean relative testis (with epididymis) weight at 1800 and 5400 ppm and mean relative kidney weight at 5400 ppm in males were observed. Microscopic examination revealed treatment-related increased pigment in the spleen at 1800 in males and at 5400 ppm in both sexes. **No adverse effects.** NOEL (M)= 36.5 mg/kg/day (600 ppm) and (F)= 135.6 mg/kg/day (1800 ppm) (based on treatment-related microscopic findings in the spleen). Acceptable (Corlett, 10/5/00).